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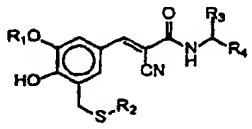
(54) Title: METHODS AND COMPOSITIONS FOR TREATING LEUKEMIA

(57) Abstract

Compounds of general formula (I), wherein R_1 is H or C1 to C3 alkyl; R_2 is aryl or $-(CH_2)_n$–aryl and n is 1 to 4; R_3 is H or CH_3; and R_4 is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole are described, as well as methods for treating cell proliferative disorders and neoplastic disorders.



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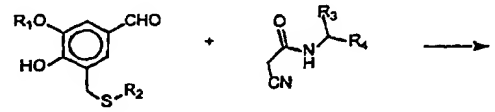

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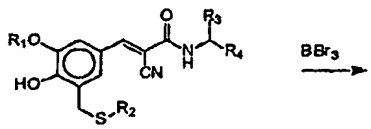

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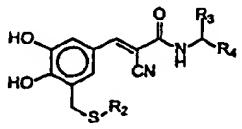

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METHODS AND COMPOSITIONS FOR TREATING LEUKEMIA

Field of the Invention

This invention relates to tyrphostins or benzylidene malononitrile compounds which are useful as antiproliferative pharmaceuticals for treating a variety of cell proliferative disorders.

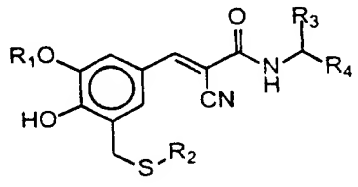
Background of the Invention

A number of tyrphostins or benzylidene malononitrile derivatives have been described which are tyrosine kinase inhibitors and are effective to inhibit cell proliferation, for example in human leukemia (United States Patents Nos. 5,217,999 and 5,773,476).

Summary of the Invention

The present invention provides a new group of tyrphostins or benzylidene malononitrile derivatives of improved effectiveness as inhibitors of cell growth.

In accordance with one embodiment, the compounds of the invention have the general formula:



wherein

R_1 is H or C1 to C3 alkyl;

R_2 is aryl or $-(CH_2)_n-$ aryl and n is 1 to 4;

R_3 is H or CH_3; and

R_4 is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole.

In accordance with a further embodiment, the compounds have the general formula I above, wherein

R_1 is H, methyl or ethyl;

R_2 is phenyl, benzyl, -$(CH_2)_2$-phenyl, -$(CH_2)_3$-phenyl or 2-thiobenzothiazole;

R_3 is H; and

R_4 is phenyl.

In accordance with a preferred embodiment, the compounds are those shown in Figures 2 to 6.

In accordance with a further embodiment, the invention provides a pharmaceutical composition comprising as active ingredient a compound of formula I above.

In accordance with a further embodiment, the invention provides a pharmaceutical composition comprising as active ingredient one of the compounds shown in Figures 2 to 6.

In accordance with a further embodiment, the invention provides a method for treating a cell proliferative disorder in a mammal comprising administering to the mammal an effective amount of a compound of formula I above.

In accordance with a further embodiment, the invention provides a method for treating a cell proliferative disorder in a mammal comprising administering to the mammal an effective amount of at least one of the compounds shown in Figures 2 to 6.

In accordance with a further embodiment, the invention provides a method for treating a neoplastic disorder in a mammal comprising administering to the mammal an effective amount of a compound of formula I above.

In accordance with a preferred embodiment, the invention provides a method for treating acute lymphoblastic leukemia (ALL) in a mammal comprising administering to the mammal an effective amount of a compound of formula I above.

In accordance with a further preferred embodiment, the invention provides a method for treating acute lymphoblastic leukemia (ALL) in a mammal comprising administering to the mammal an effective amount of at least one of the compounds shown in Figures 2 to 6.

Summary of the Drawings

Figure 1 shows in schematic form a process for synthesising the compounds of the invention. R_1 is C1 to C3 alkyl; R_2 is aryl or $-(CH_2)_n$-aryl, where n is 1 to 4; R_3 is H or CH_3; and R_4 is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole.

Figures 2 to 6 show some examples of the compounds of the invention.

Figure 7 shows the inhibitory effect (expressed as colony formation/1.5 x 10^5 cells) of several compounds of the invention on growth of G2 ALL cells.

Figure 8 shows the inhibitory effect of several compounds of the invention on growth of G2 ALL cells.

Figures 9, 10, 11 and 12 show the effect of compounds AG 1977, AG 1978, AG 2009 and AG 2010 respectively on the growth of normal BM cells, as indicated by three different assays.

Figure 13 shows inhibition of G2 ALL cells by various concentrations of the compounds of the invention.

Figure 14 shows a dose response curve of G2 ALL cell inhibition and AG 2009 concentration.

Figure 15 shows inhibition C1 ALL cells by various concentrations of AG 1977 and 1978.

Figure 16 shows inhibition of A1 ALL cells by various concentrations of the compounds of the invention.

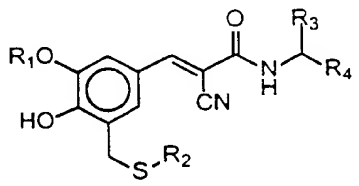
Figure 17 shows inhibition of blast cells by several compounds of the invention.

Detailed Description of the Invention

A number of tyrphostins, including the compound α-cyano-3,4-dihydroxy-cinnamal benzylamide (AG 490), have previously been shown to inhibit the growth of leukemia cells, and to be useful as active ingredients in pharmaceutical compositions for treating leukemia.

The present inventors have found that a new group of substituted benzylidene malononitriles have unexpectedly improved efficacy for treatment of cell proliferative diseases. For example, the inventors have shown that the tyrphostins described herein gave complete suppression of proliferation of human acute lymphoblastic leukemia (ALL) cells and of pre-B ALL blast cells without significantly affecting normal bone marrow cells, as described in the examples.

The compounds of the present invention have the formula:



I

wherein

R_1 is H or C1 to C3 alkyl;

R_2 is aryl or $-(CH_2)_n$- aryl and n is 1 to 4;

R_3 is H or CH_3; and

R_4 is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole.

The compounds of the invention are prepared by the process shown schematically in Figure 1. The required aldehydes (a) are available commercially or can be synthesised as previously described (Gazit et al., (1993), J. Med. Chem., v. 36, p. 3556). Benzyl cyano acetamide (b) is synthesised as described previously (Gazit et al., (1991), J. Med. Chem., v. 34, p. 1896).

A preferred group of compounds are the compounds shown in Figures 2 to 6.

The compounds of the invention may be used to treat a variety of neoplastic disorders, including leukemia, lymphomas, metastatic carcinomas and other forms of cancer. Leukemias which may be treated include B-lineage Acute Lymphoblastic Leukemia (ALL), such as the aggressive Philadelphia$^+$ leukemia, and acute myelocytic leukemia and juvenile myelo monocytic leukemia; lymphomas which may be treated include B-lineage Burkitt's lymphoma and Non-Hodgkin's lymphomas, such as the Ki-1 positive anaplastic large cell lymphomas.

The compounds of the invention may also be used to reduce or inhibit cell growth in a variety of cell proliferative disorders such as inflammatory disorders, allergic disorders, autoimmune diseases and graft rejection situations in which cell growth suppression, and preferably T cell growth suppression, is desired.

The compounds of the invention may also be used to inhibit the activity of Jak2 kinase. They may therefore be used to treat any disorder associated with increased or undesired Jak2 kinase activity.

The compounds of this invention may be used in the form of the free base, in the form of salts and as hydrates. All forms are within the scope of the invention. Acid addition salts may be formed and provide a more convenient form for use; in practice, use of the salt form inherently amounts to

use of the base form. The acids which can be used to prepare the acid addition salts include preferably those which produce, when combined with the free base, pharmaceutically acceptable salts, that is, salts whose anions are non-toxic to the animal organism in pharmaceutical doses of the salts, so that the beneficial properties inherent in the free base are not vitiated by side effects ascribable to the anions. Although pharmaceutically acceptable salts of the basic compounds are preferred, all acid addition salts are useful as sources of the free base form even if the particular salt per se is desired only as an intermediate product as, for example, when the salt is formed only for the purposes of purification and identification, or when it is used as an intermediate in preparing a pharmaceutically acceptable salt by ion exchange procedures.

Pharmaceutically acceptable salts within the scope of the invention include those derived from the following acids; mineral acids such as hydrochloric acid, sulfuric acid, phosphoric acid and sulfamic acid; and organic acids such as acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, cyclohexylsulfamic acid, quinic acid, and the like.

Compounds may be examined for their efficacy in inhibiting cell growth in cell proliferation assays such as those described herein.

In accordance with the methods of the invention, the described tyrphostins may be administered to a leukemia patient in a variety of forms depending on the selected route of administration, as will be understood by those skilled in the art. The compositions of the invention may be administered orally or parenterally, the latter route including intravenous and subcutaneous administration. Parenteral administration may be by continuous infusion over a selected period of time.

The active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsules, or it may be compressed into tablets, or it may

be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like.

The active compound may also be administered parenterally or intraperitoneally. Solutions of the active compound as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersion can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersion and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists.

The therapeutic compounds of this invention may be administered to a mammal alone or in combination with pharmaceutically acceptable carriers, as noted above, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration and standard pharmaceutical practice.

Tyrphostins are administered initially in a suitable dosage to provide a blood level of about 10 μM. The dosage may be adjusted as required, depending on the clinical response.

An alternate therapeutic approach is to obtain bone marrow or peripheral blood cells containing stem cells from patients with leukemia or lymphoma and to treat these cells ex vivo with a tyrphostin of the invention to purge or kill leukemia or lymphoma cells present with minimal inhibition of normal stem cells. The treated cells are washed to remove excess tyrphostin and returned to the patient.

For such ex vivo treatment of cells over a short period, for example around 5 hours, higher doses of tyrphostin may be used than for long term in vivo therapy; for example, concentrations of 50μM or higher may be used.

Examples

The Examples are described for the purposes of illustration and are not intended to limit the scope of the invention.

Example 1 - Synthesis of Compounds

The compounds of the invention were synthesised generally by the process shown schematically in Figure 1.

Compound AG 1946: N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-methylene thiobenzyl phenyl) acrylamide

$R_1 = CH_3$, R_2 = benzyl, R_3 = H, R_4 = phenyl

(a) 207 mg 0.72mM 4-hydroxy-3-methoxy-5-methylene thiobenzyl benzaldehyde, 130 mg 0.75 mM N-benzyl cyano acetamide and 10 mg β-alanine in 25 mL ethanol were refluxed 3 hours. Evaporation and trituration with dichloromethane-hexane gave 305 mg yellow-green solid, 95% yield, mp-135°.

NMR (acetone d_6) δ 8.17(1H,s,vinyl), 7.70(1H,d,J=2.1 Hz), 7.57(1H,d,J=2.1 Hz), 7.3(10H,m), 4.60(2H,s), 3.93(3H,s), 3.78(2H,s), 3.74(2H,s).

Compound AG 1977: N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiobenzyl phenyl) acrylamide

R_1 = H, R_2 = benzyl, R_3 = H, R_4 = phenyl

(b) To 450 mg of product from (a) in 30 mL dichloromethane was added 0.5 mL BBr_3. After stirring 1 hour at room temperature, water

was added and the reaction extracted with ethyl acetate. Evaporation and trituration with dichloromethane-hexane gave 340 mg, 78% yield, yellow solid, mp-195°.

NMR (acetone d_6) δ 8.08(1H,s,vinyl), 7.62(1H,d,J=2.2 Hz), 7.3(11H,m), 4.58(2H,s), 3.78(2H,s), 3.75(2H,s).

MS m/e-430(M^+,16%), 175(100%).

Compound AG 1951: N-benzyl-2-cyano-3-(3'-ethoxy-4'-hydroxy-5'-methylene thiophenyl phenyl) acrylamide

$R_1 = CH_2CH_3$, R_2 = phenyl, R_3 = H, R_4 = phenyl

(c) 500 mg 1.74mM 3-ethoxy-4-hydroxy-5-methylene thiophenyl benzaldehyde, 310 mg 1.78 mM N-benzyl cyano acetamide and 25 mg β-alanine in 40 mL ethanol were refluxed 4 hours. Evaporation and trituration with hexane gave 730 mg yellow solid, 95% yield, mp-108°.

NMR (acetone d_6) δ 8.10(1H,s,vinyl), 7.70(1H,d,J=2.2 Hz), 7.53(1H,d,J=2.2 Hz), 7.3(10H,m), 4.58(2H,d,J=6.0 Hz), 4.26(2H,s), 4.18(2H,q,J=7.0 Hz), 1.42(3H,t,J=7.0 Hz).

Compound AG 1978: N-benzyl-2-cyano-3-(3',4-dihydroxy-5'-methylene thiophenyl phenyl) acrylamide

R_1 = H, R_2 = phenyl, R_3 = H, R_4 = phenyl

(d) To 200 mg of product from (c) in 30 mL dichloromethane was added 0.4 mL BBr_3. After stirring 1 hour at room temperature water was added and the reaction extracted with ethyl acetate. Evaporation and trituration with dichloromethane-hexane gave 91 mg, 47% yield, yellow solid, mp-175°.

NMR (acetone d_6) δ 8.01(1H,s,vinyl), 7.63(1H,d,J=2.2 Hz), 7.3(11H,m), 4.58(2H,s), 4.26(2H,s).

MS m/e- 416(M^+,16%), 309(12), 263(32), 196(37), 175(100%).

Compound AG 2007: N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide

$R_1 = CH_3$, $R_2 = CH_2CH_2Ph$, $R_3 = H$, R_4 = phenyl

(e) 400 mg 1.3 mM 4-hydroxy-3-methoxy-5-methylene thiophenethyl benzaldehyde, 240 mg 1.38 mM N-benzyl cyano acetamide and 20 mg β-alanine in 40 mL ethanol were refluxed 4 hours. Evaporation and trituration with dichloromethane- hexane gave 450 mg yellow solid, 88% yield, mp-102°.

NMR ($CDC1_3$) δ 8.25(1H,s,vinyl), 7.62(1H,d,J=2.2 Hz), 7.3(11H,m), 4.61(2H,d,J=6.0 Hz), 3.96(3H,s), 3.81(2H,s), 2.80(4H,m).

Compound AG 2009: N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide

$R_1 = H$, $R_2 = CH_2CH_2Ph$, $R_3 = H$, R_4 = phenyl

(f) To 320 mg of product from (e) in 25 mL dichloromethane was added 0.3 mL BBr_3. After stirring 1 hour at room temperature water was added and the reaction extracted with ethyl acetate. Evaporation and trituration with dichloromethane-hexane gave 110 mg, 35% yield, yellow solid, mp-153°.

NMR (acetone d_6) δ 8.09(1H,s,vinyl), 7.63(1H,d,J=2.2 Hz), 7.3(11H,m), 4.58(2H,s), 3.85(2H,s), 2.80(4H,m).

Compound AG 2008: N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide

$R_1 = CH_3$, $R_2 = CH_2CH_2CH_2Ph$, $R_3 = H$, R_4 = phenyl

(g) 800 mg 2.5 mM 4-hydroxy-3-methoxy-5-methylene thiopropylphenyl benzaldehyde, 430 mg, 2.5 mM N-benzyl cyano acetamide and 20 mg β-alanine in 40 mL ethanol were refluxed 4 hours. Evaporation and trituration with $Cc1_4$ gave 760 mg yellow solid, 63% yield, mp-78°.

NMR ($CDC1_3$) δ 8.25(1H,s,vinyl), 7.62(1H,d,J=2.2 Hz), 7.3(11H,m), 4.61(2H,d,J=6.0 Hz), 3.96(3H,s), 3.81(2H,s), 2.69(2H,t,J=6.0 Hz), 2.50(2H,t,J=6.0 Hz), 1.90(2H,quint.,J=6.0 Hz).

Compound AG 2010: N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide

R_1 = H, R_2 = $CH_2CH_2CH_2Ph$, R_3 = H, R_4 = phenyl

(h) To 660 mg of product from (g) in 25 mL dichloromethane was added 0.6 mL BBr_3. After stirring 1 hour at room temperature water was added and the reaction extracted with ethyl acetate. Evaporation and trituration with dichloromethane-hexane gave 610 mg, 95% yield. yellow solid, mp-138°.

NMR (acetone d_6) δ 8.17(1H,s,vinyl), 7.63(1H,d,J=2.2 Hz), 7.3(11H,m), 4.58(2H,s), 3.80(2H,s), 2.69(2H,t,J=6.0 Hz), 2.52(2H,t,J=6.0 Hz), 1.90(2H,quint.,J=6.0 Hz).

Compound AG 1976: N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene (2'-thiobenzothiazole) phenyl) acrylamide

R_1 = H, R_2 = 2-thiobenzothiazole, R_3 = H, R_4 = phenyl

(i) 330 mg, 1 mM, 4-hydroxy-3-methoxy-5-methylene (2-thio-benzothiazole) benzaldehyde, 180 mg, 1.03 mM, N-benzyl cyano acetamide and 15 mg β-alanine in 20 ml ethanol were refluxed 4 hours. Cooling and filtering gave 460 mg, 95% yield, yellow solid.

(j) To 200 mg, 0.4 mM, solid from (i) in 30 ml dichloromethane was added 0.4 ml BBr_3. After stirring 1 hour at room temperature, water and 3 ml HCl was added and the reaction extracted with ethyl acetate. Evaporation and trituration with dichloromethane-hexane gave 40 mg, 20% yield, bright yellow solid, mp-225%.

NMR Acetone d_6 δ 8.07(1H,s,vinyl), 7.95(2H,m), 7.6 7.1(9H,m), 4.60(2H,s), 4.48(2H,d,J=5.9 Hz).

Example 2 - Inhibition of Colony Formation - Acute Lymphoblastic Leukemia (ALL) Cell Lines

Inhibition of colony formation was studied by methods described previously (Kamel-Reid et al., (1992), Leukemia, v. 6, pp. 8-17; Meydan et al., (1996), Nature, v. 379, pp. 645-648).

ALL cell lines A1 (at $8x10^5$ cells/ml), C1 (at $4x10^4$ cells/ml) and G2 (at $1.15x10^6$ cells/ml) were plated in 1 ml volumes, in the absence of exogenous growth factors, into 35 mm petri dishes (Nunc, Gibco) containing alpha MEM (Gibco) plus 10% FCS (Cansera Rexdale, Ont.) in 0.9% (vol/vol) methylcellulose (Fluka, Switzerland). Cultures were set up at 37°C with 5% CO_2 in a humidified atmosphere and 10 uM of a selected tyrphostin was added. Colonies consisting of more than 20 cells were counted at 12 days (A1), 5 days (C1) and 14 days (G2) using an inverted microscope. The results with G2 are shown in Figure 7. Similar results were obtained with A1 and C1.

Example 3 - Effect on Bone Marrow Cells

Compounds showing inhibition of ALL colony formation were examined for their effect on normal bone marrow cells using a modified CFU-GEMM clonogenic assay.

The assay was performed according to Fauser and Messner (1978), Blood, v. 52, pp. 1243-8, and Messner and Fausser (1980) , Blut, v. 41, pp. 327-333, with some variations. In brief, heparinized bone marrow cells were layered over Percoll (Pharmacia Fine Chemical, Piscataway N.J.) at a density of 1.077 gm/ml and centrifuged at 400 g at 4°C for 10 min. to remove neutrophils and RBCs. The fractionated bone marrow cells at $2x10^5$ cells/ml were cultured in IMDM (OCI, Toronto) containing 0.9% (vol/vol)

methylcellulose supplemented with 30% FCS (Cansera Rexdale, Ont.) or normal human plasma, a cocktail of cytokines consisting of G-CSF (10 ng/ml, Amgen), IL-3 (40 U/ml, Immunex), MGF (50 ng/ml, Immunex), Erythropoietin (2u/ml, Epprex) or TPO (10 ng/ml, Amgen) and $5x10^{-5}$ 2-mercaptoethanol. The culture mixture was plated in 1 ml volumes into 35 mm petri dishes and incubated at 37°C with 5% CO_2 in a humidified atmosphere with concentrations of tyrphostin up to 40 μm. The results are shown in Figures 9 to 12.

The BFU-E's (erythroid colonies) and the CFU-GEMM (mixed colonies) exhibited inhibition at and above 25μM (Fig. not shown), while the CFU-C's (granulocytes, monocytes and macrophages) showed a dramatic increase of colony proliferation peaking at 25μM and a reduction by 50μM (Fig. not shown). AG 2010 showed significant inhibition at 40μM, while the remaining compounds showed mild to significant inhibition of erythroid and mixed colonies followed by the myeloid population at 20μM.

Example 4 - Inhibition of ALL Cells

Various concentrations of tyrphostins were tested for inhibition of ALL cells in the clonogenic assay described in Example 2. Compounds AG 1977, 1978, 2007, 2008, 2009 and 2010 were tested against ALL cell lines A1, C1 and G2 in doses ranging from nanomolar to micromolar values. The results are shown in Figures 8, 13, 15 and 16.

AG 2009 demonstrated the most potent clonogenic inhibition, in a dose responsive manner, against G2 cells (Fig. 13). It showed a greater than 50% inhibition at a dose of 16nM and a differential therapeutic index of greater than 2 logs in a survival curve (Fig. 14) of normal BM and G2 colonies.

Example 5 - Inhibition of Blast Cells

The compounds were further tested in an ALL blast colony assay, against bone marrow samples from two patients with pre –B ALL phenotype based on their FAB classification.

The ALL blast colony assay was performed as described previously (Estrov, Z. et al., (1988), Cancer Res., v. 48, p. 5901) with some modifications. Briefly, heparinized bone marrow cells were layered over Percoll (density 1.077 g/l; Pharmacia Fine Chemicals Piscataway, N.J.) and centrifuged (400g) for 10 minutes at 4°C to remove neutrophils and RBC's. The collected interphase fraction was further enriched for lymphoblasts before plating by using a magnetic cell separator, mini MACS, with separation column (Miltenyi Biotec Inc., 1250 Oakmead Park, Sunnyvale, Ca). With this procedure, ALL blasts were specifically isolated from the marrow mononuclear cell fraction using directly labelled MACS CD19 Microbeads - monoclonal anti-human CD19 (Mouse IgG1, Kappa- Miltenyi Biotec Inc.) and/or indirect magnetic cell labeling using primary biotinylated antibody- (mouse anti-human CD10 monoclonal antibody, Caltag Laboratires, Ca.) and Streptavidin Microbeads (Miltenyi Biotech Inc.). The resulting cell population was composed of 99% lymphoblasts. The positively sorted cells were then cultured at $2x10^5$ cells/ml in alpha MEM (GIBCO) containing 0.9% (vol/vol) methylcellulose supplemented with 10% FCS (Cansera, Rexdale, Ont.). Irradiated autologous leukemic blasts were used as feeder cells (at $3x10^5$ cells/ml). Cytokines, normally used, were deleted so that only spontaneous proliferation was evident.

The culture mixture was plated into 35mm petri dishes (Nunc, GIBCO) containing 1ml volumes and incubated at 37°C with 5% CO_2 in a humidified atmosphere. Colonies containing more than 20 cells were scored, using inverted microscope, at 5-7 days.

In both cases, significant inhibition of blast colonies was observed (See for example, Figure 17).

Example 6 - Inhibition of Jak2 Kinase Activity

Compounds chosen on the basis of their ability to inhibit the growth (colony formation) of the pre-B leukemia cell line G2 were tested for inhibition of Jak2 kinase.

Jak2 kinase was immunoprecipitated from a 1% Triton-X100 lysate of $10x10^6$ G2 cells. An *in vitro* kinase assay was performed on the immunoprecipitated Jak2 in the presence or absence of varying concentrations of the compounds AG 1977, AG 1978, AG 2009 and AG 2010.

Stock solutions of 100mM tyrphostin were made in 100% DMSO and further dilutions made in 10% DMSO. Control kinase assays carried out in the presence of DMSO concentrations of 5-30% alone were unaffected by its presence.

Initial experiments were done with tyrphostin concentrations of 0.1, 1.0 and 10μM. These concentrations had no affect on the kinase activity of Jak2. Using higher concentrations, 5, 25 and 50μM, inhibition could be seen, as shown in Table 1 below. These results were obtained by scanning autoradiographs of the kinase assays.

TABLE 1

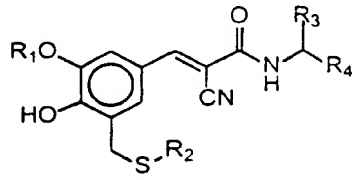
%inhibition of kinase activity

	Concentration		
Tyrphostin	5μM	25μM	50μM
AG 1977	23	14	15
AG 1978	10	13	46
AG 2009	0	0	8
AG 2010	5	5	39

The present invention is not limited to the features of the embodiments described herein, but includes all variations and modifications within the scope of the claims.

WE CLAIM:

1. A compound of the general formula:



I

wherein

R_1 is H or C1 to C3 alkyl;

R_2 is aryl or $-(CH_2)_n$- aryl and n is 1 to 4;

R_3 is H or CH_3; and

R_4 is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole.

2. A compound in accordance with claim 1 wherein

R_1 is H, methyl or ethyl;

R_2 is phenyl, benzyl, $-(CH_2)_2$-phenyl, $-(CH_2)_3$-phenyl or 2-thiobenzothiazole;

R_3 is H; and

R_4 is phenyl.

3. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-methylene thiobenzyl phenyl) acrylamide.

4. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(3'-ethoxy-4'-hydroxy-5'-methylene thiophenyl phenyl) acrylamide.

5. A compound in accordance with claim1 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene (2'-thiobenzothiazole) phenyl) acrylamide.

6. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiobenzyl phenyl) acrylamide.

7. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiophenyl phenyl) acrylamide.

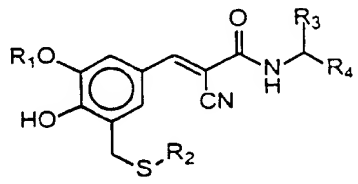
8. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.

9. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide.

10. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.

11. A compound in accordance with claim 1 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide.

12. A pharmaceutical composition comprising a compound of the formula:



I

wherein

R_1 is H or C1 to C3 alkyl;

R_2 is aryl or $-(CH_2)_n$- aryl and n is 1 to 4;

R_3 is H or CH_3; and

R_4 is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole

and a pharmaceutically acceptable carrier.

13. A pharmaceutical composition in accordance with claim 12 comprising a compound of formula I, wherein

R_1 is H, methyl or ethyl;

R_2 is phenyl, benzyl, $-(CH_2)_2$-phenyl, $-(CH_2)_3$-phenyl or 2-thiobenzothiazole;

R_3 is H; and

R_4 is phenyl.

14. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-methylene thiobenzyl phenyl) acrylamide.

15. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3'-ethoxy-4'-hydroxy-5'-methylene thiophenyl phenyl) acrylamide.

16. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene (2'-thiobenzothiazole) phenyl) acrylamide

17. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiobenzyl phenyl) acrylamide.

18. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiophenyl phenyl) acrylamide.

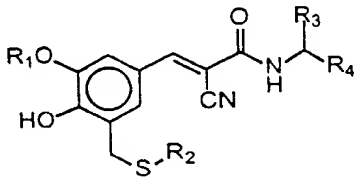
19. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.

20. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide.

21. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.

22. A pharmaceutical composition in accordance with claim 12 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide.

23. A method for treating a neoplastic disorder in a mammal comprising administering to the mammal an effective amount of a compound of the formula:



I

wherein

R_1 is H or C1 to C3 alkyl;

R_2 is aryl or $-(CH_2)_n$- aryl and n is 1 to 4;

R_3 is H or CH_3; and

R_4 is substituted or unsubstituted phenyl, pyridyl, thiophene, furan, indole, pyrrole, thiazole or imidazole.

24. A method in accordance with claim 23 comprising administering an effective amount of a compound of formula I wherein

R_1 is H, methyl or ethyl;

R_2 is phenyl, benzyl, $-(CH_2)_2$-phenyl, $-(CH_2)_3$-phenyl or 2-thiobenzothiazole;

R_3 is H; and

R_4 is phenyl.

25. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-methylene thiobenzyl phenyl) acrylamide.

26. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3'-ethoxy-4'-hydroxy-5'-methylene thiophenyl phenyl) acrylamide.

27. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene (2'-thiobenzothiazole) phenyl) acrylamide.

28. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiobenzyl phenyl) acrylamide.

29. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-methylene thiophenyl phenyl) acrylamide.

30. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.

31. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(4'-hydroxy-3'-methoxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide.

32. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thioethyl phenyl) phenyl) acrylamide.

33. A method in accordance with claim 23 wherein the compound is N-benzyl-2-cyano-3-(3',4'-dihydroxy-5'-(methylene thiopropyl phenyl) phenyl) acrylamide.

34. A method in accordance with any one of claims 23 to 33 wherein the neoplastic disorder is a lymphoma, a leukemia or a metastatic carcinoma.

35. A method in accordance with any one of claims 23 to 33 wherein the neoplastic disorder is Acute Lymphoblastic Leukemia.

36. A method for treating a cell proliferative disorder in a mammal comprising administering to the mammal an effective amount of a compound in accordance with any one of claims 1 to 11.

37. A method in accordance with claim 36 wherein the cell proliferative disorder is selected from the group consisting of an inflammatory disorder, an allergic disorder, an autoimmune disease or graft rejection.

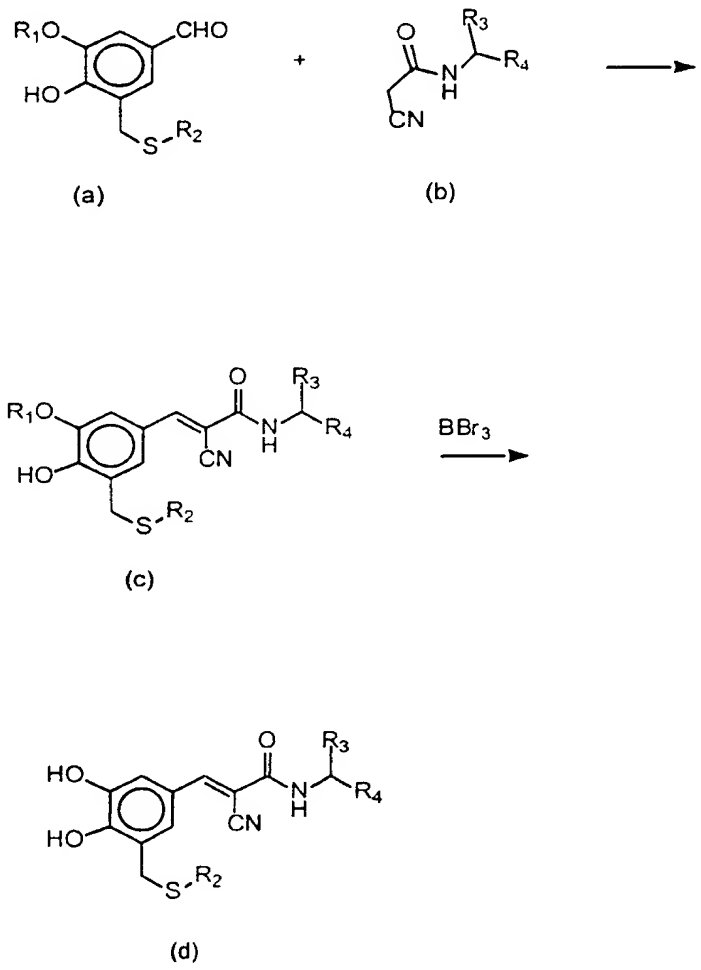
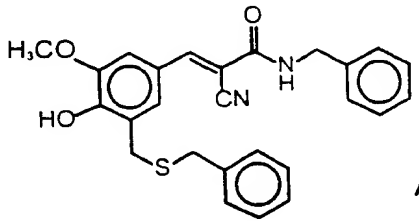
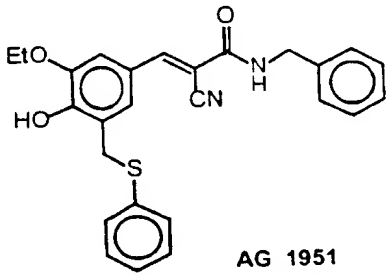


Figure 1

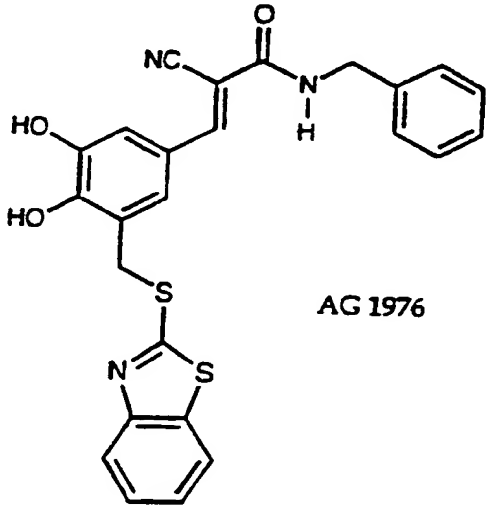


AG 1946

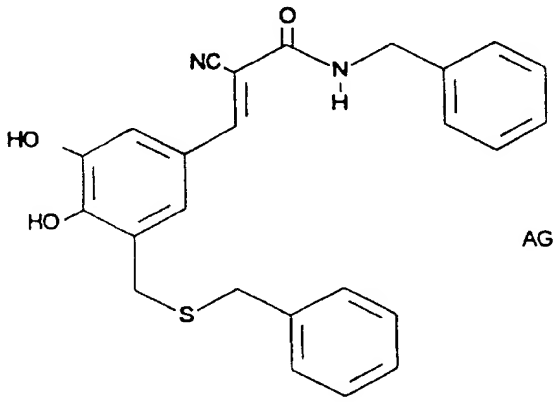


AG 1951

Figure 2

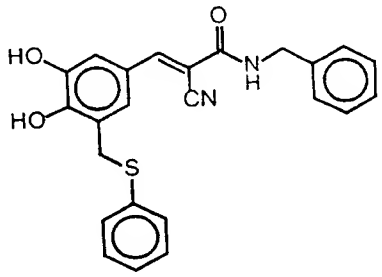


AG 1976

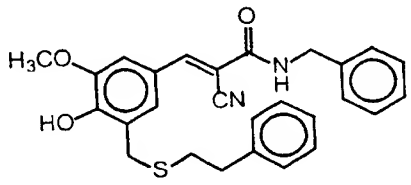


AG 1977

Figure 3

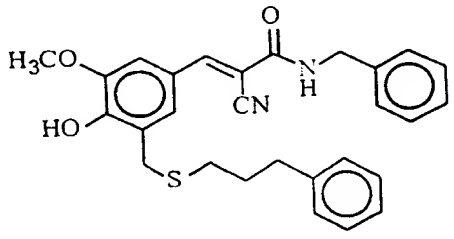


AG 1978



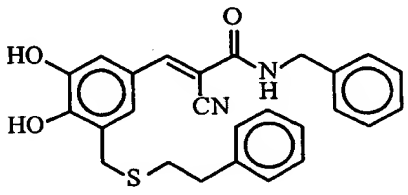
AG 2007

Figure 4

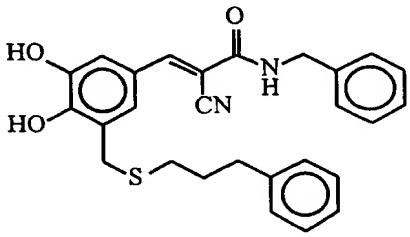


AG 2008

Figure 5

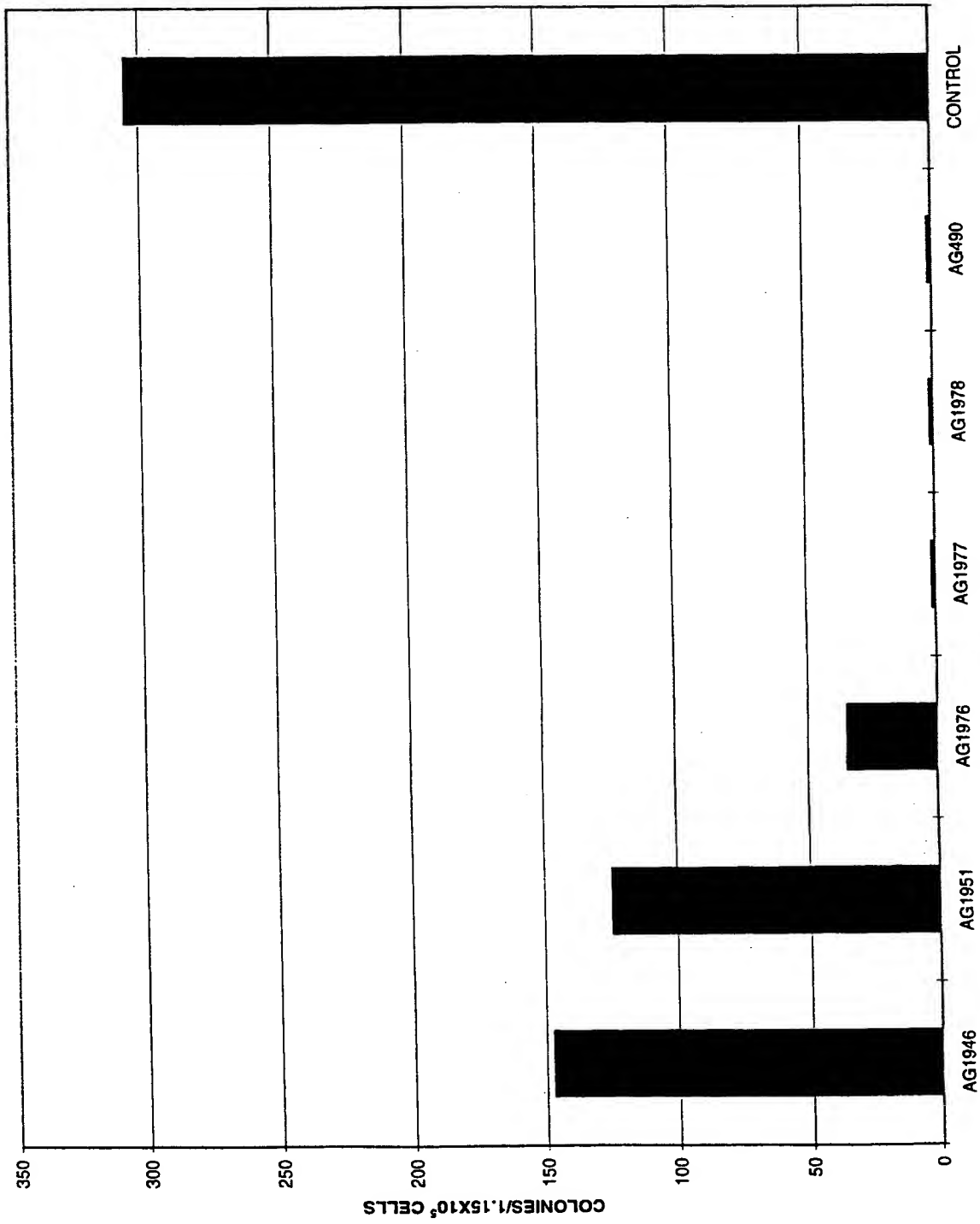


AG 2009



AG 2010

Figure 6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AG1978
AG1977
AG1976
AG1951
AG1946
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100
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300
350
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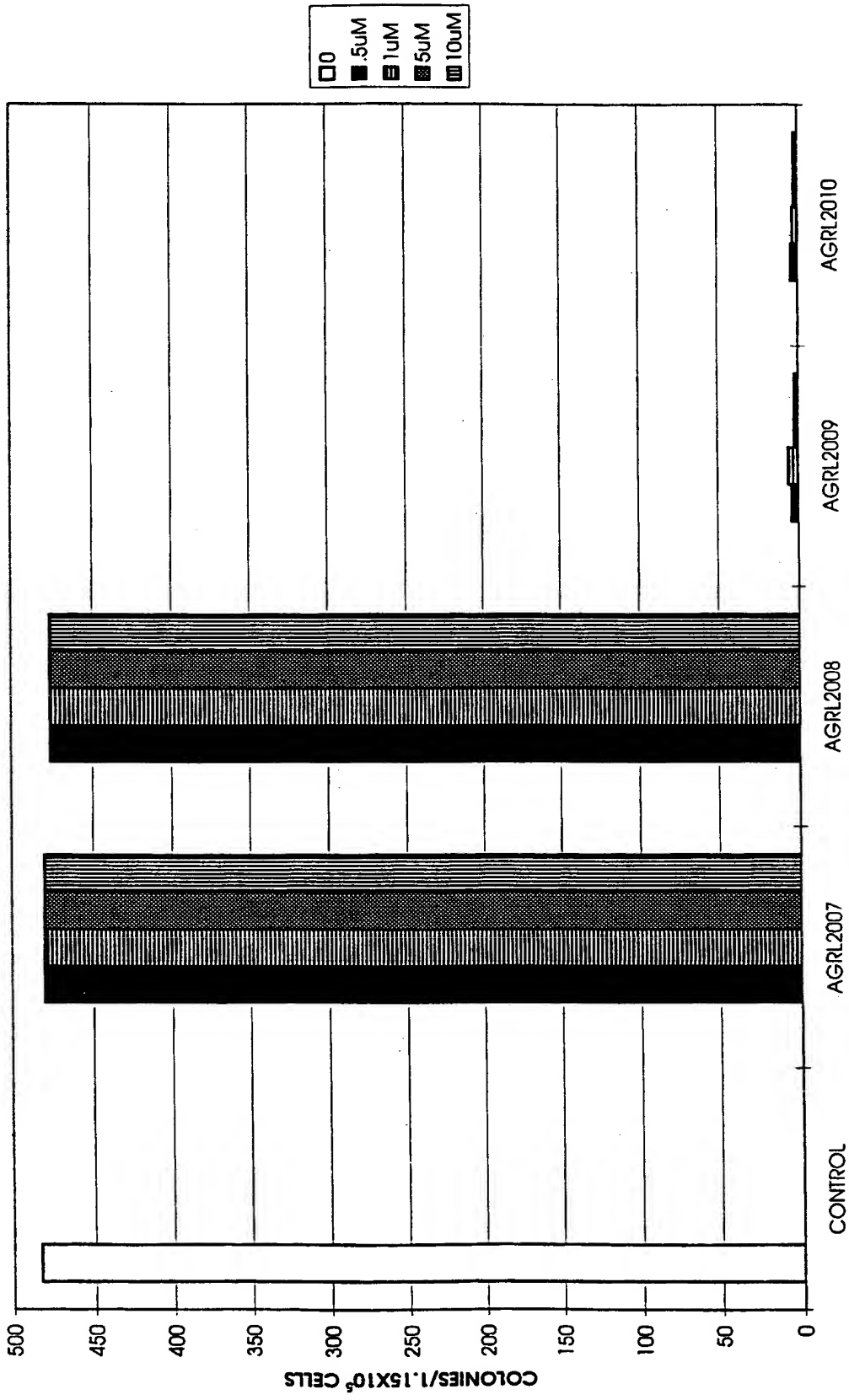
Figure 7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1uM
5uM
10uM
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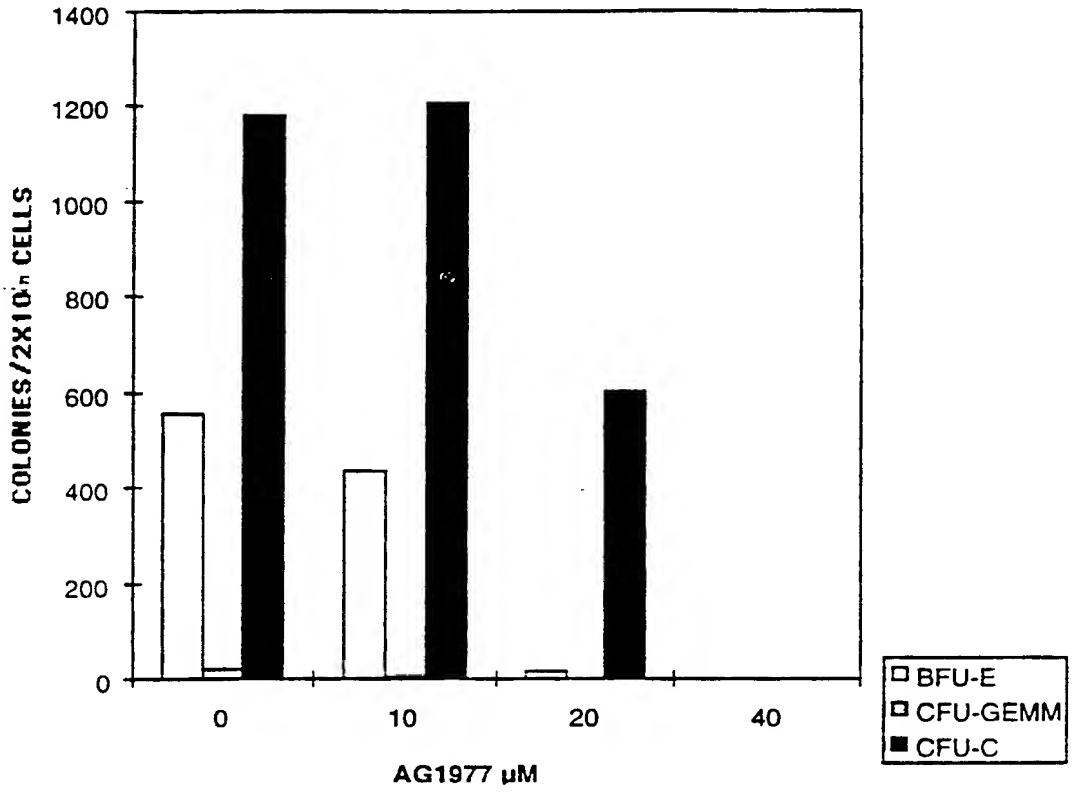
200
150
100
50
0
CONTROL
AGRL2007
AGRL2008
AGRL2009
AGRL2010
TYRPHOSTINS

Figure 8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1200
1000
800
600
400
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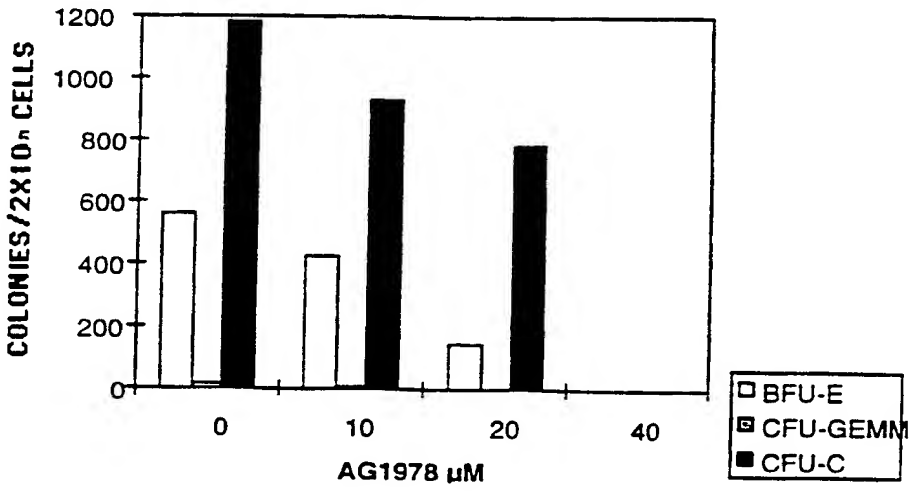
AG1977 µM
BFU-E
CFU-GEMM
CFU-C

Figure 9


COLONIES/2X10n CELLS
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200
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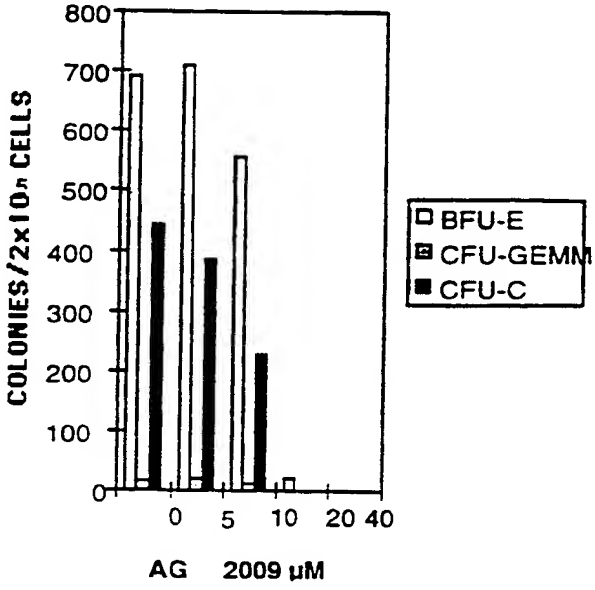
AG1978 µM
BFU-E
CFU-GEMM
CFU-C

Figure 10


800
700
600
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400
300
200
100
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COLONIES/2x10⁵ CELLS
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5
10
20
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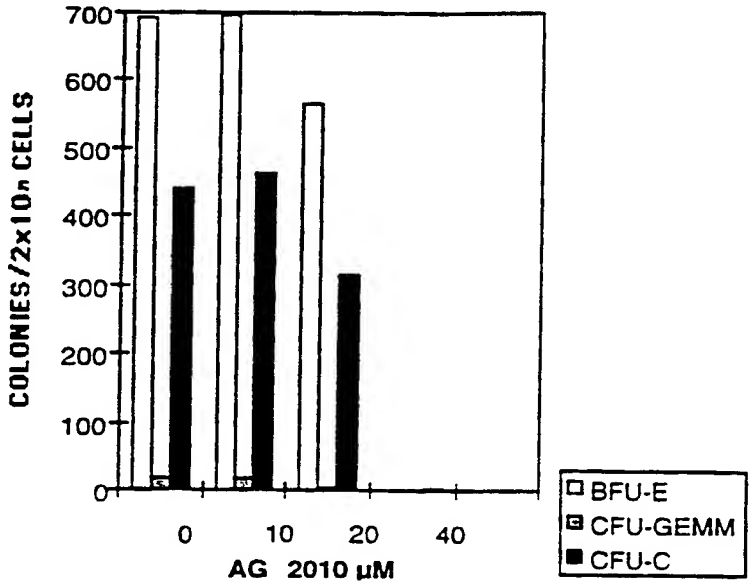
AG 2009 µM
BFU-E
CFU-GEMM
CFU-C

Figure 11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COLONIES/2x10n CELLS
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10
20
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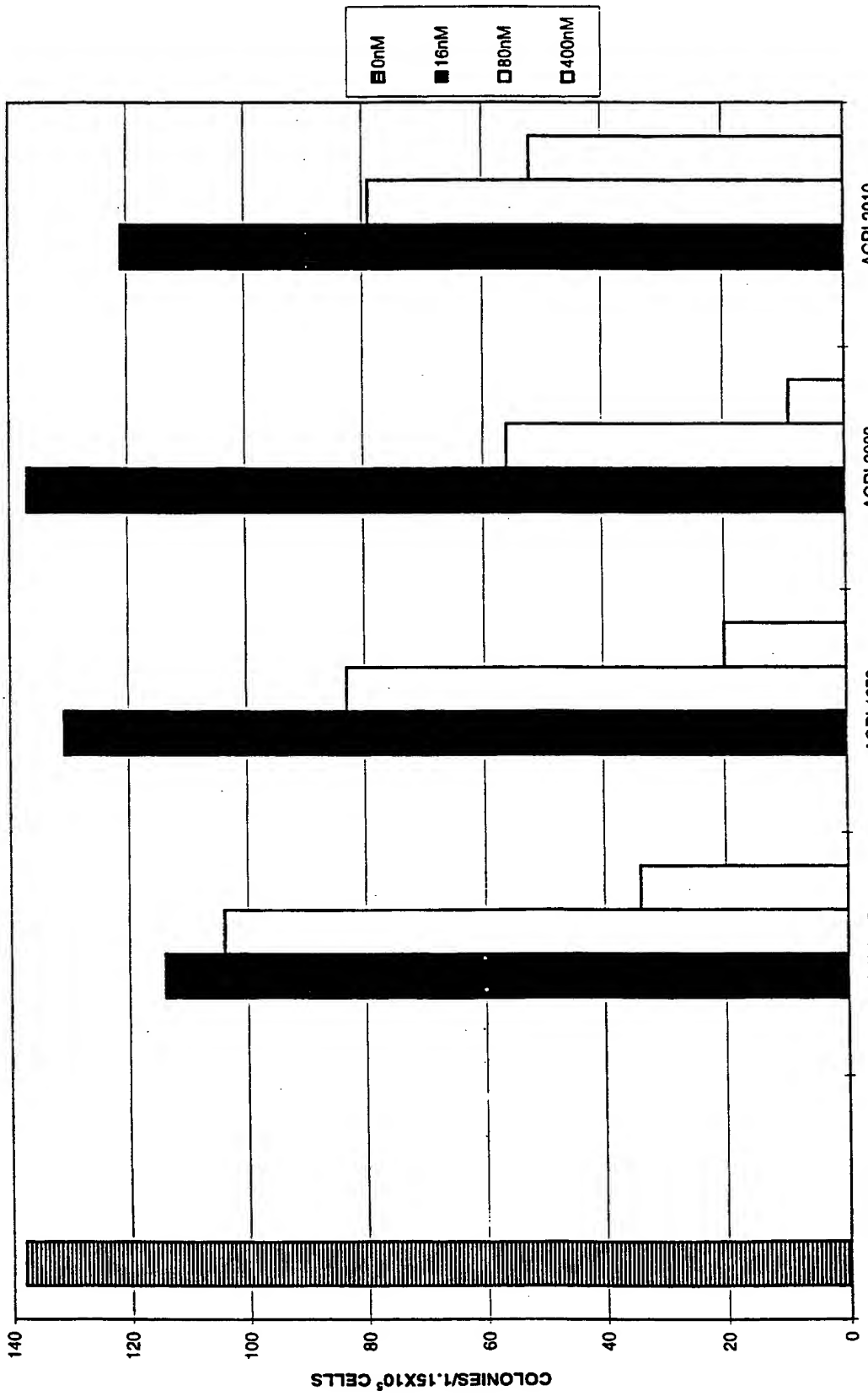
AG 2010 µM
BFU-E
CFU-GEMM
CFU-C

Figure 12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80nM
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AGRL2010
AGRL2009
AGRL1978
AGRL1977
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60
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COLONIES/1.15X10⁵ CELLS

Figure 13

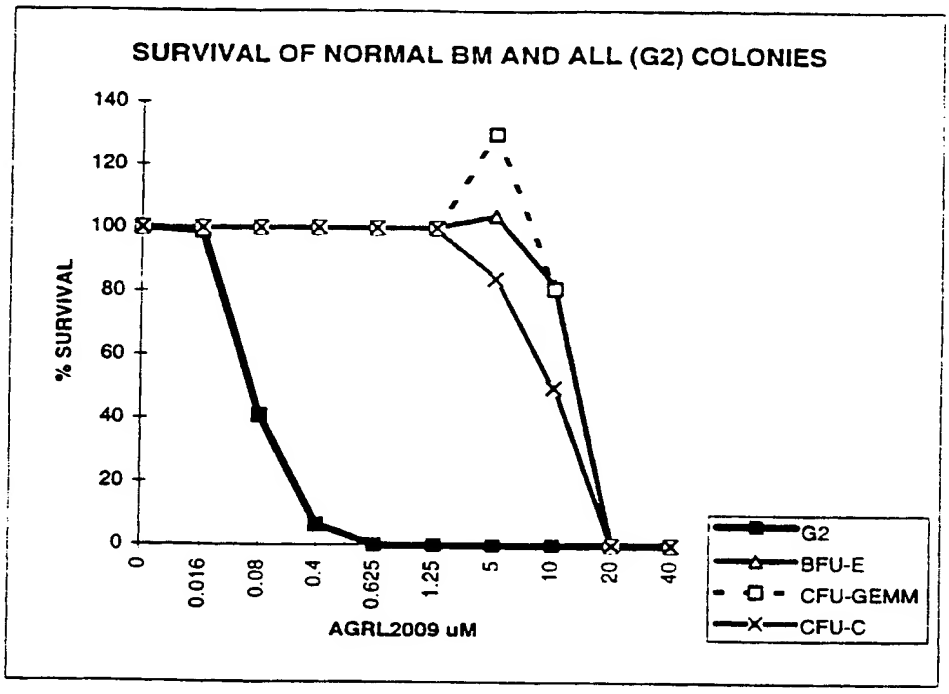
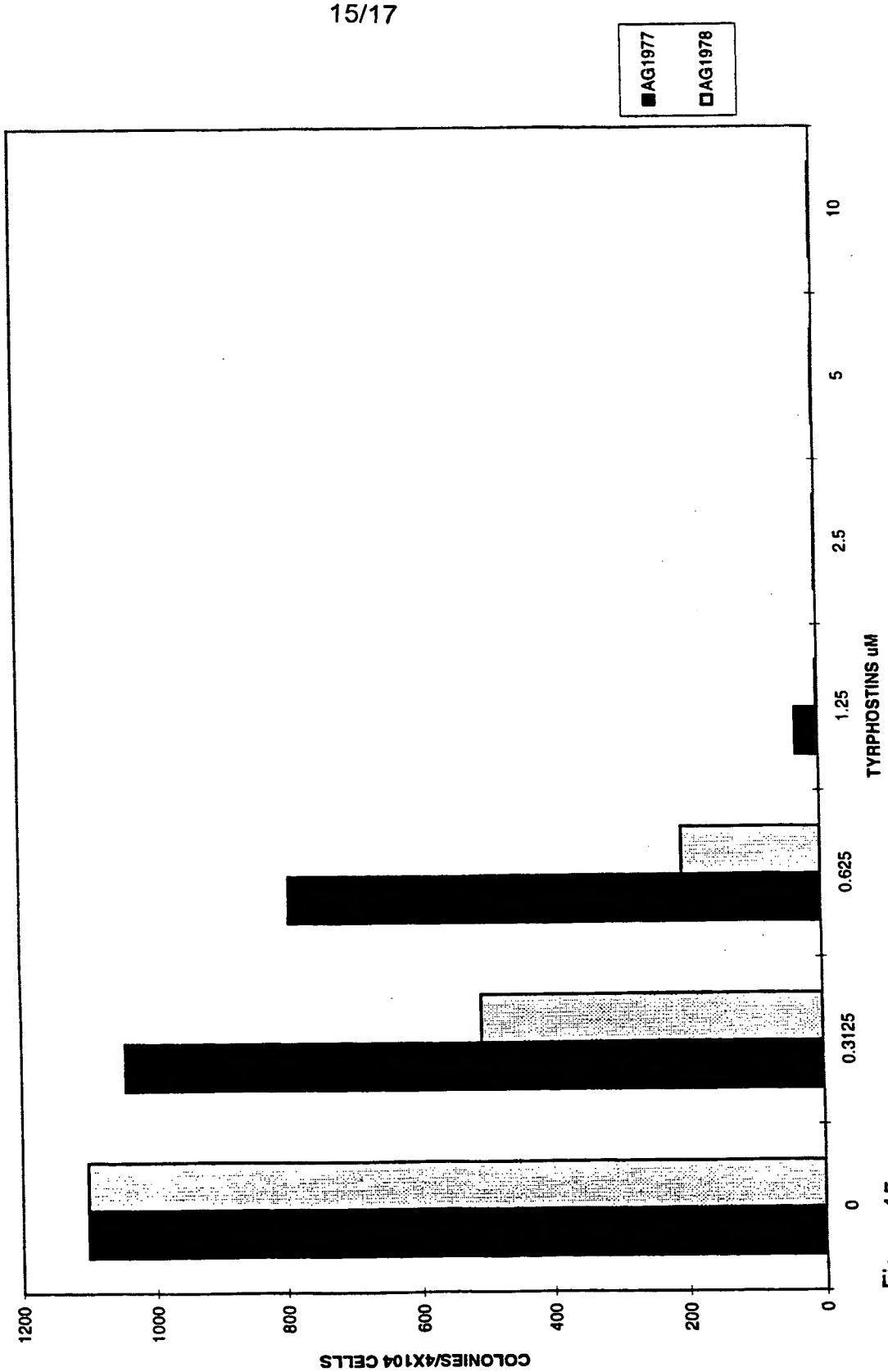

SURVIVAL OF NORMAL BM AND ALL (G2) COLONIES
% SURVIVAL
140
120
100
80
60
40
20
0
0
0.016
0.08
0.4
0.625
1.25
5
10
20
40
AGRL2009 uM
G2
BFU-E
CFU-GEMM
CFU-C

Figure 14


AG1977
AG1978
TYRPHOSTINS uM
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0.3125
0.625
1.25
2.5
5
10
COLONIES/4X104 CELLS
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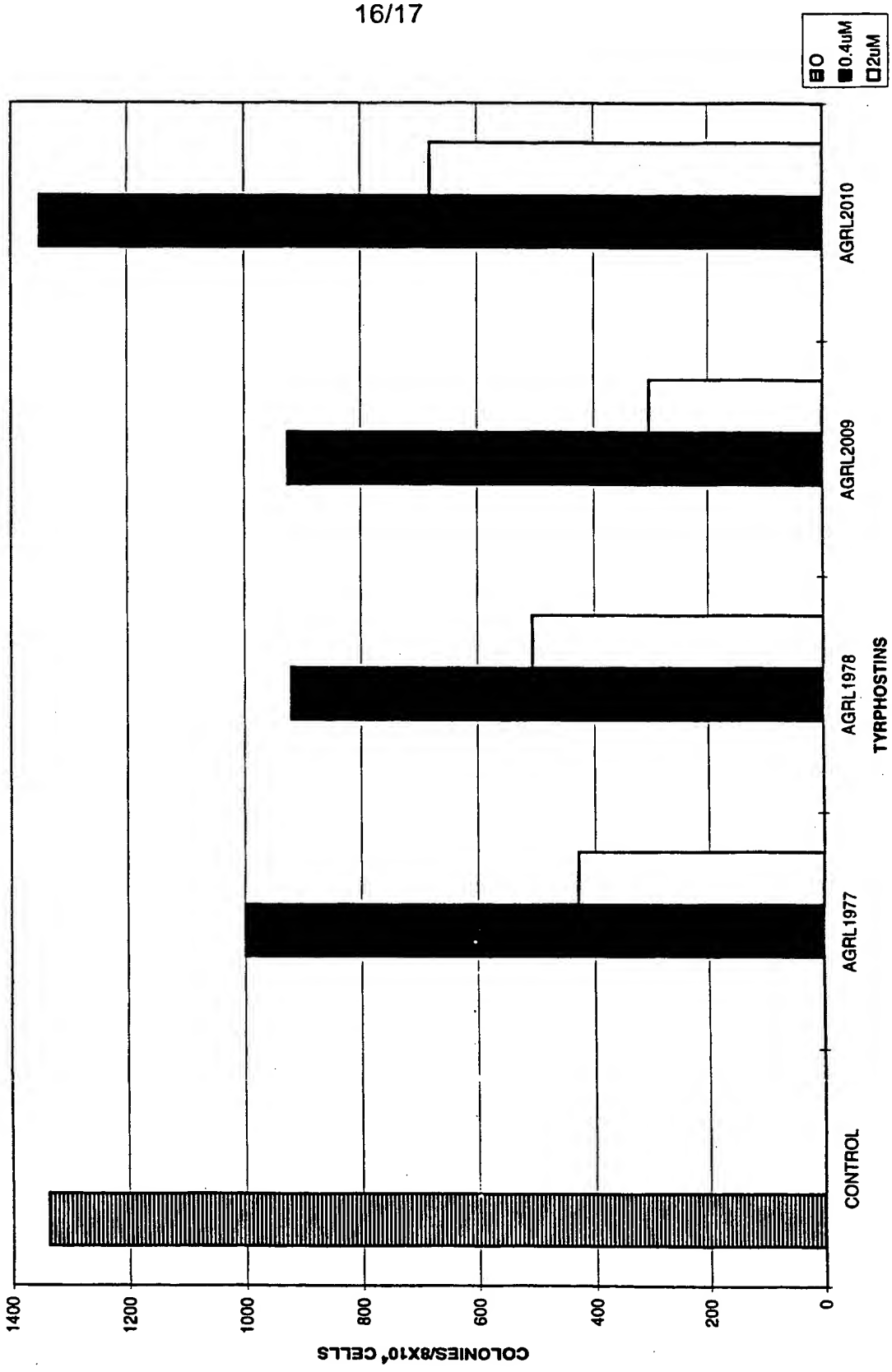
200
400
600
800
1000
1200

Figure 15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0.4uM
2uM
AGRL2010
AGRL2009
AGRL1978
AGRL1977
CONTROL
TYRPHOSTINS
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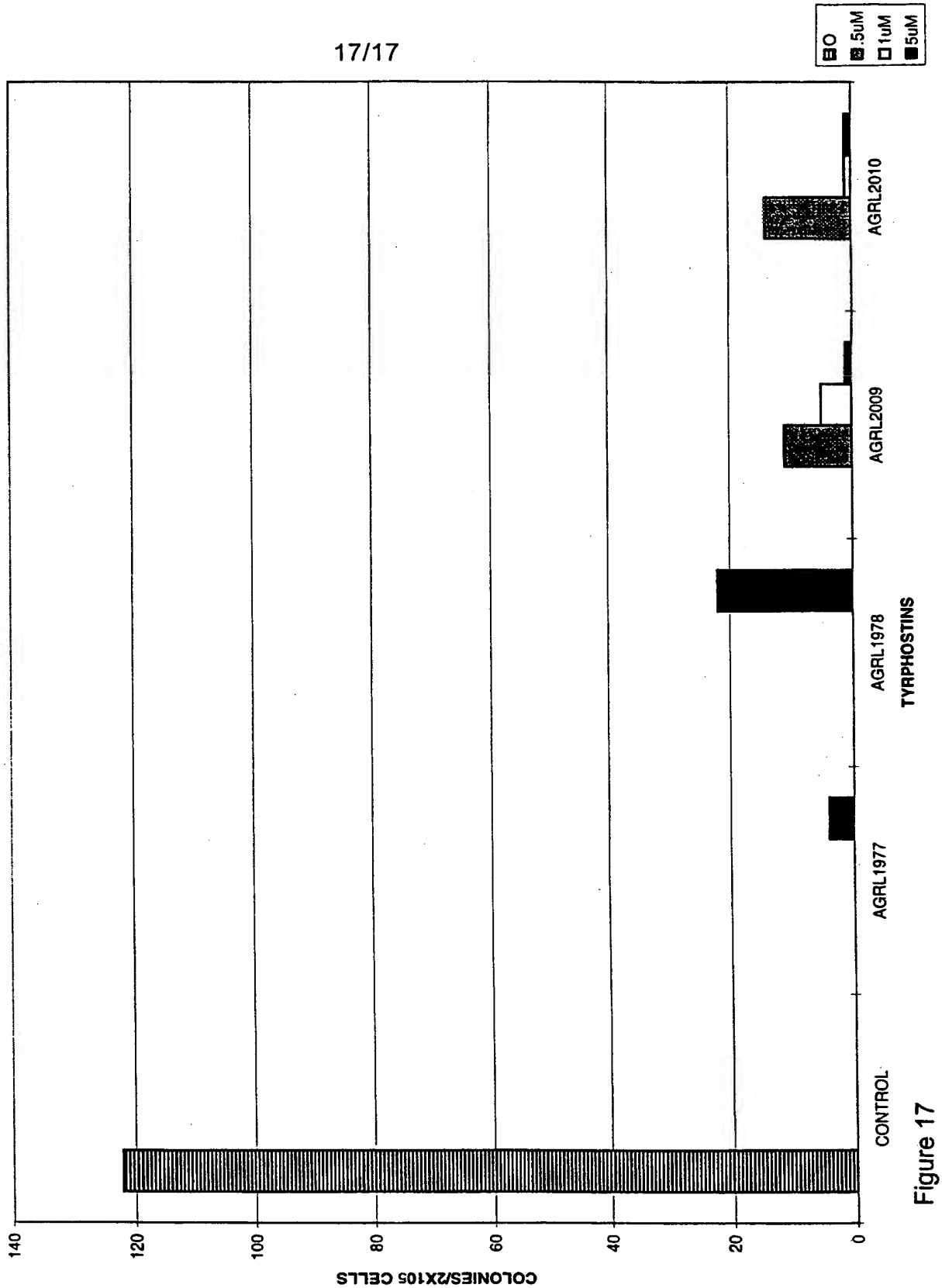
1200
1000
800
600
400
200
0
COLONIES/8X10⁴ CELLS

Figure 16


0
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1uM
5uM
AGRL2010
AGRL2009
AGRL1978
AGRL1977
CONTROL
TYRPHOSTINS
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120
100
80
60
40
20
0
COLONIES/2X105 CELLS

Figure 17

INTERNATIONAL SEARCH REPORT

Inter[redacted] Application No
PCT/CA 00/00266

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C323/60 C07D277/74 A61K31/277 A61K31/428 A61P35/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 773 476 A (A. GAZIT, ET AL.) 30 June 1998 (1998-06-30) cited in the application the whole document ---	1,12,23
A	A. GAZIT, ET AL.: "Tyrphostins. 2. Heterocyclic and alpha-substituted benzylidenemalononitrile tyrphostins as potent inhibitors of EGF receptor and ErbB2/neu tyrosine kinases" JOURNAL OF MEDICINAL CHEMISTRY, vol. 34, no. 6, 1 June 1991 (1991-06-01), pages 1896-1907, XP000472938 American Chemical Society, Washington, DC, US ISSN: 0022-2623 table III --- -/--	1,12,23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 May 2000	13/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	English, R

2

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter[redacted] Application No
PCT/CA 00/00266

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 26260 A (YISSUM RESEARCH DEVELOPMENT) 24 November 1994 (1994-11-24) claims 1,2,4	1,12,23

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter[redacted]l Application No
PCT/CA 00/00266

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5773476 A	30-06-1998	US 5789427 A	04-08-1998
		AU 2096895 A	25-09-1995
		WO 9524190 A	14-09-1995
WO 9426260 A	24-11-1994	AU 6910994 A	12-12-1994
		ZA 9403305 A	30-01-1995